# SYNTHESIS AND CHARACTERIZATION OF COBALT(II) COMPLEXES OF LINCOMYCIN AND ITS ACETYLATED DERIVATIVE

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## ABSTRACT

Co (II) complexes of lincomycin and the tetraacetylated derivative of lincomycin (CoLin and CoAcL respectively, where Lin is lincomycin and AcL is tetra-O-acetyl-Lincomycin) have been synthesized and isolated in solid state. The intermediate compounds and the isolated complexes were characterized by their solubility, melting point, FT-IR, $^{1}H$ and $^{13}C$ NMR spectra. The complexes are sparingly soluble in water but insoluble in organic solvents except in hydroxylic solvents such as methanol. The FTIR and NMR spectra of both complexes gave broad peaks being paramagnetic species and provide evidence for complex formation with peaks corresponding to lincomycin present in both FT-IR and NMR spectra of the complexes. The structures of the complexes were assigned based on the only possible coordination mode for lincomycin and is in agreement with their FTIR and NMR spectra. Detailed spectroscopic characterization of the complexes by FT-IR, and $^{1}H$ and $^{13}C$ NMR and fluorescence quenching of Ethidium Bromide (EB) bound DNA by CoLin are presented and discussed.

## INTRODUCTION

Lincomycin and clindamycin belong to the lincosaminide class of antibiotics which are widely used as broad spectrum antibiotics against Gram-positive bacteria in human and veterinary medicine. The first lincosamide to be discovered was lincomycin isolated from *Streptomyces linconensis* (Hoeksema *et al*., 1986). Lincomycin, at low concenterations, inhibits protein synthesis in gram-positive bacteria without interfering with DNA and RNA synthesis (Josten and Allen, 1964). Specifically, lincomycin acts on the 50S subunit of the ribosomes of a Gram-positive organism but not on the corresponding subunit of a Gram-negative organism (Chang *et al*., 1966). However, resistance to lincomycin by various species of *Staphylococcus* including *Staphylococcus aureus* BM4611 (Leclercq *et al*., 1987) and the biochemical mechanism of lincosaminide inactivation by this species (Brisson-Noel *et al*., 1998) have been reported.

The interactions in aqueous solution of palladium with lincomycin (Yi *et al*., 2008) and clindamycin (Yia *et al*., 2009) and the analytical applications of 1:1 palladium chelates of the two compounds obtained in solution have been investigated while the solution chemistry and dynamics of Cu (II) complexation of lincomycin has also been studied by proton and carbon-13 NMR (Gaggelli *et al*., 2002). The oxidative cleavage activity of the complex specie produced in aqueous solution was demonstrated and attributed to be a fairly efficient promoter of $^{\bullet}OH$ generation (Gaggelli *et al*., 2002).

While the solution chemistry of lincomycin with copper and palladium have been demonstrated, the search of literature revealed that no complex of lincomycin has been obtained in solid state. It is, therefore, noteworthy to obtain metal complexes of lincomycin in solid state, to characterize and to explore them for different biological applications. The afore-mentioned uses of lincosamides and their metal complexes, the therapeutic properties of cobalt, and DNA binding and cleavage abilities of transition metal complexes in general were the motivation behind this piece of research work. To this end, homoleptic cobalt complexes containing the antimicrobial

ligand lincomycin and its acetylated derivative were synthesized and the propensity of the obtained cobalt-lincomycin binding to DNA was examined.

The rationale behind this work is to take advantage of the unique properties of these complexes to apply them in DNA binding, DNA cleavage and in antimicrobial therapy such as antibacterial and antiplasmodial activities for which the parent compounds are indicated. This is in response to our continuous search for metal-containing therapeutic agents because of their proven biological potentials. (Obaleye *et al*., 2016; Abosede *et al*., 2016)

## EXPERIMENTAL

All chemicals used for syntheses were of analytical grade and were used as received. The chemicals and their sources were as follows: $CoCl_2.6H_2O$, ethidium bromide (EB), acetic anhydride, pyridine, sodium sulphate, potassium hydrogen phosphate ($K_2HPO_4$), potassium dihydrogen phosphate ($KH_2PO_4$), tris(hydroxymethyl) aminomethane hydrochloride (Tris HCl) and Calf thymus DNA sodium salt (CT DNA) were obtained from SRL, India. Lincomycin hydrochloride and its monohydrate salt were obtained from Drugfield pharmaceuticals, Sango Otta, Nigeria. The solvents methanol, ethanol, dichloromethane, chloroform, acetone, diethyl ether, benzene, acetonitrile, dimethyl formamide (DMF) and dimethyl sulphoxide (DMSO) were from S. D. Fine Chemicals Limited, India. The deuterated solvents $D_2O$ (99.9%), $CDCl_3$ (99.9%) and DMSO-$d_6$ (99.5%) were obtained from Aldrich Chemical Co. (U.S.A.).

Infrared spectra were recorded in a range 4,000–400 $cm^{-1}$ on Shimadzu FTIR-8400 at Department of Chemistry, Savitribai Phule University of Pune, India and on Perkin Elmer 400 ATR-FTIR at Rhodes University, South Africa on samples pressed as KBr pellets. Melting points were taken on a Jenway analytical instrument and were uncorrected at STEP-B, Laboratory, Department of Chemistry, University of Ilorin, Nigeria. $^1H$ NMR, $^{13}C$ NMR and COSY spectra were performed on Varian Mercury 300 MHz spectrometer at the Savitribai Phule Department of Chemistry, University of Pune, India. The reported chemical shifts were against TMS in ppm.

### Synthesis of Lincomycin Free Base

Lincomycin free base was prepared by following the procedure of Herr and Slomp (1967). A 5 g of lincomycin hydrochloride monohydrate was dissolved in 85 mL of water (pH=5). NaOH (5 mol) was added in drops to adjust the pH of the solution to 10. The solution was extracted three times with chloroform (85 mL portions) using 500 ml separating funnel. The chloroform extract was dried over sodium sulphate and evaporated under reduced pressure while adding 20 ml distilled water, 5 mL portion twice and then 10 mL portion once. The resultant aqueous solution was freeze-dried: the aqueous solution was first kept in freezer overnight to frozen and the frozen solid was kept in a lyophilizer, automatic mode: -48 $^oC$, 0.05 millitorr to freeze-dry. The freeze-dried product was kept in vacuum desiccator to finally dry. FT-IR (KBr, $\nu/cm^{-1}$): 3352, 3329, 3288, 2956, 2920, 1645, 1518, 1448, 1379, 1305, 1236, 1209, 1087, 1053, 993, 902, 866, 802, 692, 634, 605, 584, 516, 470.

### Synthesis of aceytelated Lincomycin (Formation of Tetra-O-acetyl-Lincomycin -AcL)

Tetra-O-acetyl-lincomycin (AcL) was prepared by following the procedure of Sztaricskai *et al* (1997), 20 mL of spectroscopic grade pyridine was placed on ice bath for about 2 hours after which 1.9979 g of lincomycin hydrochloride monohydrate (2.51 mmol) was added followed by 20 mL of acetic anhydride and the solution kept for 38 hours. The resulting solution was poured onto 50 mL cold water, extracted with 100 ml and 50 ml portions of chloroform. The chloroform extract was washed with 10% aqueous acetic acid (50 mL twice), water (50 mL twice) and 2% aqueous sodium hydrogen carbonate (50 ml twice). The chloroform solution was then dried with sodium sulphate and allowed to stand at room temperature to obtain white precipitated product. Melting point: 50 $^oC$. FT-IR (KBr, $\nu/cm^{-1}$): 3475, 3313, 2955, 2929, 2872, 2848, 2785, 1749

(νC-O, Ac), 1681 (amide I), 1506 (amide II), 1446, 1431, 1369, 1315, 1230, 1176, 1114, 1066, 1043, 1010, 949, 910, 875, 775, 709, 671, 648, 619, 609, 528, 482, 459, 416.

$$\boldsymbol{Lin} + \mathbf{Ac_2O} \xrightarrow{py} \boxed{\mathbf{AcL}}$$

Lin = lincomycin hydrochloride, $Ac_2O$ = acetic anhydride, AcL = acetylated lincomycin py = pyridine.

**Equation 1: Synthesis of acetylated lincomycin (AcL)**

**Synthesis of CoLin**

0.231 g (0.5 mmol) of lincomycin hydrochloride was dissolved in acetone and 0.1 mL of triethyl amine was added. The solution was heated for complete dissolution of lincomycin, cooled and 0.0822 g (0.5 mmol) of Cobalt nitrate was added. The solution was stirred for 8 hours and the yellow precipitate obtained was filtered and purified by extracting with chloroform before drying in desiccator. Calculated: C, 36.85; H, 6.18; N, 4.77. Found: C, 37.15; H, 7.25; N, 4.62. UV-Vis ($H_2O$, nm):274, 341, 370, 726, 768. FT-IR (KBr, ν/$cm^{-1}$): 3529, 3489, 3452, 3375, 3338, 3290, 3200, 3074, 3022, 2955, 2920, 2866, 2677, 1656, 1564, 1411, 1357, 1261, 1205, 1141, 1105, 1041, 962, 869, 802, 644, 464.

**Synthesis of CoAcL**

CoAcL was synthesized and purified in a similar way with CoLin using equimolar proportions of cobalt nitrate and acetylated lincomycin (AcL) in place of lincomycin. FT-IR (KBr, ν/$cm^{-1}$): 3553, 3265, 2958, 2874, 1745, 1687, 1641, 1558, 1446, 1431, 1371, 1319, 1230, 1168, 1112, 1070, 1043, 1012, 950, 910, 840, 775, 715, 621, 607, 553, 528, 424.

**Fluorescence measurements**

Competitive Binding Fluorescence Measurements of CoLin: The apparent binding constants ($K_{app}$) of the cobalt-lincomycin complex were determined by a fluorescence spectral technique using ethidium bromide (EtBr)-bound CT DNA solution in phosphate buffer. The changes in fluorescence intensities at 600 nm (510 nm excitation) of EtBr bound to DNA were recorded with an increasing amount of the complex concentration. Concentrations of DNA and EtBr were 20 μM in all ca

## RESULTS AND DISCUSSION

Two homoleptic cobalt complexes of lincomycin and acetylated lincomycin have been synthesized and isolated in solid state. The isolated complexes were characterized by FTIR spectroscopy and the coordination mode of lincomycin was assigned based on the only possible coordination mode for lincomycin as previously reported (Yi A-E *et al.*, 2008; Yi A *et al*., 2009; Gaggelli E., *et al.*). The two complexes are slightly soluble in water but insoluble in organic solvents such as acetone, ethyl acetate, chloroform except in hydroxylic solvents such as methanol and ethanol. The NMR spectra of the complexes are not well resolved being paramagnetic cobalt (II) complexes. The spectra of the complexes support complex formation with peaks corresponding to lincomycin ligand present in the spectra of the complexes. The presence, of metal-ligand bond vibrations as well as shifts in the lincomycin stretching frequencies in the obtained complexes, proves the formation of metal complexes.

The band at 1749 $cm^{-1}$ in the spectrum of acetylated lincomycin (AcL) is due to the acetyl blocking group in the molecule. This band is also present in the CoAcL complex at 1743 $cm^{-1}$ showing that the acetyl group is not coordinated to cobalt. Diagnostic bands for the CoLin complex appear at 1564 and 1656 $cm^{-1}$ which are respectively amide II and amide I stretching frequencies of lincomycin. These bands are in the same region in both lincomycin and CoLin,

indicating that the amide C = O is not involved in coordination in CoLin complex. The presence of alcohol and cyclic ester C - O stretch in lincomycin, CoLin and CoAcL spectra further confirms that carbonyl group is not participating in coordination in all compounds. The weak amine N-H stretch which appeared at 2576 $cm^{-1}$ in lincomycin spectrum is not present in both CoLin and CoAcL complexes. This confirms that amine nitrogen is participating in coordination to cobalt in both complexes.

The proposed structures of the complexes are presented in Figure 1 while Table 1 summarizes the assignment of the infrared absorption bands of the CoLin complex. The assigned coordination of the complex is similar to the assigned coordination mode for literature report for the complexation of $Cu^{II}$ -lincomycin in water solution.

Table 1. Selected of FT-IR absorption bands for Lincomycin (Lin) and its metal complex (wave number in $cm^{-1}$)

| **Lincomycin** | **CoLin** | **CoAcL** | **Assignment** |
|---|---|---|---|
| 3533, 3487, 34,52, 3375, 3338, 3288, 3201 | 3529, 3489, 3452, 3375, 3338, 3290, 3200 | | Alcohol OH and secondary amide N-H stretch |
| | 2955, 2920, 2866, | | C-H aliphatic C-H aromatic |
| 2576 w | - | - | Amine N-H stretch |
| 1656 | 1656, | 1687, 1641 | Secondary Amide C=O stretch |
| 1566 | 1564 | 1558 | Secondary Amide II absorption |
| 1105, 1076, 1041 | 1105, 1041 | 1112, 1070, 1043 | Alcohol and cyclic ester C-O stretch |

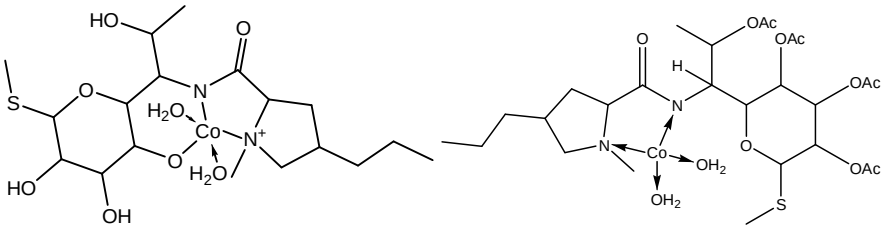


Fig. 1: tentative structure of Cobalt-lincomycin (CoLin) and CoAcL (Ac is acetyl group)

EB does not fluoresce, but the emission intensity of EB in the presence of DNA is greatly enhanced, due to strong intercalation between the adjacent DNA base pairs. It was previously reported that this enhanced fluorescence can be quenched, at least partly, by the addition of a second molecule (Selvi *et al.*, 2005; Kawade *et al.*, 2011). The extent of fluorescence quenching of EB bound to DNA by a molecule is usually utilized to determine the extent of binding between the second molecule and DNA. The emission spectra of EB bound to DNA in the absence and presence of the CoLin complex is given in Figure 2. The addition of the complex to DNA, pretreated with EB, causes an appreciable reduction in emission intensity than that observed in the absence of the complex. This indicates that the complex competes with EB in binding to DNA and also implying that its affinity to DNA is strong.

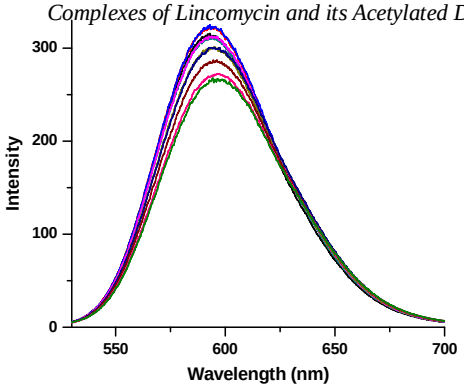


Fig. 2: Fluorescence quenching of ethidium bromide bound DNA by CoLin in Tris buffer: CoLin 0-700 μM; [DNA], [EtBr]=20 μM; [DNA]/[Co]=0-35

## CONCLUSION

In the present study new cobalt complexes of lincomycin and acetylated licomycin have been synthesized and characterized with the aid of FT-IR and UV-Vis spectroscopy, solubility and melting point. The propensity of the CoLin complex to bind to DNA has also been investigated by fluorescence experiments. These experiments demonstrate that the cobalt (II) complex can bind DNA in preference to ethidium bromide. The decrease in ethidium bromide emission intensity observed showed that the mode of binding of the complex to DNA is by intercalation. Although drug development is not the focus of this research, it helps to understand the mechanisms of action of the parent drug and will further in the design of new diagnostic and therapeutic products involving lincomycin or its derivative.

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